Biosensors for Water Environment Monitoring: A Postprint

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Abstract

Biosensors utilize the specific recognition interactions between biomolecules to enable detection of biological and chemical targets, holding significant application value across various fields. Addressing the needs of water environment monitoring, this article investigates and summarizes the research and development of biosensors, primarily elaborating on enzyme, immunological, DNA, tissue, and microbial biosensors, as well as their application research in the field of water environment monitoring.

Full Text

Biosensors for Water Environment Monitoring

Preamble

Biosensors utilize specific recognition between biomolecules to detect biological and chemical targets, holding significant application value across numerous fields. This article addresses the needs of water environment monitoring, surveying and summarizing the research and development of biosensors. It primarily elaborates on enzyme, immune, DNA, tissue, and microbial biosensors, along with their application studies in water environment monitoring.

Keywords: biosensor, water pollution monitoring, enzyme, immune, DNA, tissue, microorganism
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With the development of industry, agriculture, and society, large volumes of industrial and agricultural wastewater and domestic sewage are discharged, leading to frequent water pollution incidents that severely damage the water resources environment upon which human survival depends. Water pollution prevention and control have become urgent priorities. Conducting water pollution

monitoring and effectively tracking water quality indicators in a timely manner constitute critical components of water pollution prevention. Traditional water pollution monitoring and analysis methods suffer from cumbersome operation procedures, long testing cycles, bulky instrumentation, and high costs, making them ill-suited for wide-area, real-time field detection and distributed online monitoring applications. There is an urgent need to develop miniaturized, low-cost, simple-to-operate, and rapid-response water environment monitoring technologies and instruments. Simultaneously, certain trace, highly toxic, and persistent pollutants, such as heavy metal ions and persistent organic pollutants, demand detection methods and technologies with ultra-high sensitivity.

Biosensors leverage specific biomolecular recognition and reactions by immobilizing biomolecular recognition elements on sensor surfaces to achieve specific identification of target analytes. Recognition elements are typically biological components (e.g., enzymes, antigens, antibodies, DNA) or biological entities themselves (e.g., cells, tissues). The biosensor transducer further converts changes in chemical or physical signals into measurable electrical signals, enabling detection of analyte concentrations. Transducers can be electrochemical, optical, thermal, piezoelectric, or magnetic [1]. Biosensors offer the advantages of good selectivity and high sensitivity. Compared with traditional biological and chemical detection instruments, biosensors feature small size, simple equipment, easy operation, rapid response, low power consumption, and low cost, facilitating on-site and rapid detection of biological and chemical substances. They hold broad application prospects in medical health, food safety, environmental monitoring, and numerous other fields.

Based on recognition elements, biosensors can be classified into enzyme sensors, immune sensors, DNA sensors, tissue sensors, microbial sensors, etc. This article reviews the application research of several different types of biosensors in water environment monitoring.

Enzyme Sensors in Water Environment Monitoring

Enzymes are a class of proteins with biological activity that catalyze specific reactions. They exhibit characteristics including fast reaction rates, high catalytic efficiency, mild reaction conditions, and high selectivity and specificity, making them widely used for preparing enzyme biosensors. Enzyme sensors enable in-situ, accurate, sensitive, and rapid detection of biochemical substances. In water pollution monitoring, enzyme sensors are commonly used for detecting organophosphorus pesticides, phenolic substances, nitrates, and heavy metal ions.

For organophosphorus pesticide detection, enzyme sensors typically utilize the inhibitory effect of organophosphorus pesticides on acetylcholinesterase activity, achieving detection by measuring current changes. In recent years, numerous novel materials for acetylcholinesterase immobilization have emerged, such as gold nanoparticles [2], silver nanowires [3], and polymer materials like micro-

gels [4]. New methods have also appeared, such as organophosphorus detection based on tapered fiber enzyme sensors [5]. Wei and Feng [6] studied an electrochemical biosensor based on acetylcholinesterase/nitrogen-doped porous carbon/boron-doped diamond electrodes. The porous structure and good biocompatibility of nitrogen-doped porous carbon provided abundant reaction sites for acetylcholinesterase immobilization, effectively maintaining enzyme activity, while nitrogen doping enhanced electrode surface conductivity and accelerated electron transfer rates. Test results showed detection ranges of 0.1–10,000 ng/L for both dichlorvos and fenitrothion, with detection limits as low as 1.50 pg/L and 4.40 pg/L, respectively.

To reduce enzyme immobilization difficulty while fully utilizing enzyme specificity, Caballero-Díaz et al. [7] developed a fluorescent nanosensor using nitrogen-doped graphene quantum dots and acetylcholinesterase as biological recognition elements for determining the insecticide fenoxycarb in river water. This sensor achieved detection through the quenching effect of enzyme products on nitrogen-doped graphene quantum dot fluorescence, eliminating the need for enzyme immobilization. Test results indicated a linear detection range of 6–70 µmol/L for fenoxycarb, with a detection limit of 3.15 µmol/L and good reproducibility.

Phenolic substances are common highly toxic pollutants in water environments, and their detection holds significant importance. Sethuraman et al. [8] developed a composite modified glassy carbon electrode based on poly(3,4-ethylenedioxythiophene)-reduced graphene oxide-iron(III) oxide-polyphenol oxidase (PEDOT-rGO-Fe_2O_3-PPO) for specific detection of catechol. The prepared composite electrode exhibited high enzyme loading capacity and fast electron transfer rates, with a linear detection range of 4$\times10^{\{-8\}–6.20\times10}\{-5\}$ mol/L for catechol and a detection limit of 7$\times10\^{}\{-9\}$ mol/L. The biosensor maintained stability for up to 75 days when stored in buffer at approximately 4°C.

Excessive nitrates in water pose health hazards, and nitrate detection occupies an important position in water quality monitoring [9]. Minami et al. [10] first reported an extended-gate organic field-effect transistor (OFET) based enzyme biosensor [Figure 1: see original paper] for nitrate detection, achieving a detection limit as low as 45 µg/L. Due to the printability, mechanical flexibility, stretchability, and disposability of OFETs, this research opens a new pathway for developing low-cost, field-deployable nitrate sensors. Ali et al. [11] designed a microfluidic impedimetric nitrate sensor based on graphene oxide (GO) nanosheets and PEDOT nanofibers (PEDOT-NF), where the PEDOT-NFs-GO composite immobilized nitrate reductase. Studies demonstrated synergistic effects between GO and PEDOT-NF. The sensor exhibited a sensitivity of 61.15 $\Omega \cdot \mathrm{L} \cdot \mathrm{mg}^{-1} \cdot \mathrm{cm}^{-2}$ in the nitrate ion concentration range of 0.44–442 mg/L, with a detection limit of 0.135 mg/L and good specificity, reliability, and reproducibility.

[Figure 1: see original paper] Nitrate biosensor based on extended-gate organic field-effect transistor [10]

Accompanying industrialization, wastewater containing heavy metal ions (Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Cr^{3+}, Hg^{2+}, etc.) is discharged in large quantities, seriously endangering the water environment and aquatic organisms. Utilizing the inhibitory effects of heavy metal ions on enzymes, sensors for heavy metal detection can be developed using enzymes such as horseradish peroxidase [12], lactate dehydrogenase [13], urease [14], and glucose oxidase [15]. The in-situ, accurate, rapid, and sensitive detection of trace mercury ions in water environments is of particular concern. Elsebai et al. [16] immobilized catalase on glassy carbon electrode surfaces using glutaraldehyde and bovine serum albumin as cross-linking agents to develop an enzyme sensor for mercury ion detection [Figure 2: see original paper], achieving trace mercury detection with a detection limit of 1.8$×10^{-11}$ mol/L and a linear range of 5×10^{-11}–5×10^{-10}$ mol/L. When applied to mercury ion determination in different water samples, the sensor demonstrated excellent selectivity for mercury ions.

[Figure 2: see original paper] Enzyme sensor for mercury ion detection [16]

Immune Sensors in Water Environment Monitoring

Immune sensors are a class of biosensors that achieve detection based on specific affinity reactions between antigens and antibodies. Antibody-based immunoassay techniques are used in environmental monitoring sensor development due to their simple operation, portability, low cost, and rapid response, currently finding applications primarily in pesticide and heavy metal ion detection [17–19].

Guo et al. [20] designed a SPR biosensor based on anti-triazophos monoclonal antibodies, which exhibited good specificity for the insecticide triazophos with a low detection limit of 0.096 ng/mL and a linear range of 0.98–8.29 ng/mL. The sensor chip could be reused up to 160 times. Belkhamssa et al. [21] modified atrazine antibodies on multi-walled carbon nanotube field-effect transistors to detect the organic pesticide atrazine based on immune reactions, achieving a detection limit of 0.001 ng/mL and a linear range of 0.001–10 ng/mL. Monerris et al. [22] prepared an electrochemical immunosensor for determining trace estrone in water samples by immobilizing anti-estrone monoclonal antibodies on glassy carbon electrode surfaces.

For heavy metal ion detection, antibodies are typically conjugated with EDTA-chelated metal ions to achieve specific recognition [19]. Shu et al. [23] utilized mouse anti-Cu^{2+}-EDTA monoclonal antibodies to capture Cu^{2+}-EDTA chelates, then employed UV radiation to degrade the immune complexes and release free Cu^{2+}. Based on the fluorescence quenching effect of Cu^{2+} on CdSe/ZnS quantum dots, high-specificity and high-sensitivity detection of Cu^{2+} was achieved. The immunosensor had a detection limit of 0.33 ng/mL. Since antibodies were used to capture Cu^{2+}, interference from other heavy metal ions on quantum dot fluorescence quenching was avoided, improving sensor selectivity. López et al. [24] designed a Cd^{2+} immunosensor based on anti-Cd^{2+}-EDTA monoclonal

antibodies, which featured a wide detection range of 0.4–2,000 µg/L and a detection limit of 0.1 µg/L.

Xing et al. [25] developed a paper-based immunosensor based on immunochromatography for simultaneous detection of five pollutants in water: heavy metals, algal toxins, antibiotics, hormones, and insecticides. Using Pb(II), microcystin-leucine-arginine (MC-LR), chloramphenicol (CAP), testosterone (T), and chlorothalonil (CTN) as representatives, five different antigens were immobilized on nitrocellulose membranes. Target analytes in samples competed with immobilized antigens for binding to monoclonal gold-labeled antibodies. The detection limits for the five substances were 4 ng/mL, 1 ng/mL, 0.1 ng/mL, 5 ng/mL, and 5 ng/mL, respectively, with a detection time of 20 minutes. This paper-based sensor provides an effective method for on-site semi-quantitative detection of water pollutants.

DNA Sensors in Water Environment Monitoring

DNA exhibits high affinity similar to antigen-antibody binding, along with high sensitivity, selectivity, stability, low toxicity, and ease of synthesis and modification, making it a commonly used recognition element in biological and chemical detection. Currently, DNA is frequently employed to detect heavy metal ions and organic pollutants such as pesticides and antibiotics in water.

The property that Hg^{2+} causes thymine (T)-rich single-stranded DNA to fold and form T-Hg^{2+}-T specific structures, thereby inhibiting DNA hybridization reactions, enables the development of DNA sensors for Hg^{2+} detection. Jia et al. [26] developed a localized surface plasmon resonance (LSPR) fiber-optic DNA sensor for Hg^{2+} detection in water. The sensor constructed gold nanoparticle core-satellite structures linked by DNA hybridization double strands to excite plasmon coupling enhancement effects. By detecting the influence of Hg^{2+} on plasmon coupling intensity and LSPR resonance wavelength during the inhibition of DNA hybridization reactions, Hg^{2+} detection was achieved with a linear range of 5–150 nmol/L and a detection limit of 3.4 nmol/L.

Zuo et al. [27] proposed a dual-color fluorescent biosensor based on WS_2 nanosheets for simultaneous detection of Hg^{2+} and Ag^{+}. The sensor utilized the fluorescence quenching capability of WS_2 nanosheets and their interaction with DNA molecules. By monitoring fluorescence intensity changes at 525 nm and 583 nm, simultaneous detection of Hg^{2+} and Ag^{+} was realized, with linear ranges of 6.0–650.0 nmol/L and 5.0–1,000.0 nmol/L, and detection limits of 3.3 nmol/L and 1.2 nmol/L, respectively.

Leveraging the property that nucleases recognize specific DNA double strands and cleave one strand, and that heavy metals can activate nucleases to hydrolyze and release single strands that can re-hybridize with other molecular beacons to trigger cyclic amplification, detection of heavy metal ions can be achieved. Zhao et al. [28] developed a fluorescent cyclic amplification system based on nucleases and deoxyribozymes for Pb^{2+} detection, achieving a detection limit of

0.1 nmol/L with good selectivity. Hong et al. [29] developed an electrochemical biosensor based on this principle for Hg^{2+} detection, with a linear range of 10–50,000 pmol/L and an extremely low detection limit of 1.6 pmol/L.

Arvand and Mirroshandel [30] designed a fluorescence enhancement aptasensor based on graphene oxide/aptamer-quantum dots (GO/aptamer-QDs) composites for detecting edifenphos fungicide (EDI). The sensor utilized fluorescence resonance energy transfer from quantum dots to graphene sheets, causing quantum dot fluorescence quenching, and the displacement effect of EDI on GO to achieve fluorescence enhancement detection of EDI. The sensor exhibited a linear detection range of 5$×10^{-4}–6×10^{-3}$ mg/L, a detection limit of 1.3$×10^{-4}$ mg/L, good reproducibility (RSD=3.9%, n=10), and excellent selectivity against other chemically similar pesticides. Zourob et al. [31] screened high-affinity, highly specific DNA aptamers for carbendazim and modified gold electrode surfaces with self-assembled monolayers to achieve specific detection of carbendazim, with a detection range of 10–10,000 ng/L and a detection limit of 8.2 ng/L. Other common pesticides such as isoproturon, atrazine, linuron, trifluralin, carbaryl, and methyl parathion did not interfere with the sensor' s detection.

Tissue Sensors in Water Environment Monitoring

In recent years, plant and animal tissues have also been employed as recognition elements in biosensors [1], particularly aquatic plants have become important research tools for environmental studies. Researchers can assess environmental conditions by controlling external environmental factors (e.g., light, heat, herbicides, heavy metals, organic pollutants) and detecting corresponding changes in plant life activities [32].

Using algae as an example, Tsopela et al. [33] designed a portable on-site herbicide detection device based on algae, consisting of an electrochemical three-electrode microfluidic platform. The device detected herbicides by exploiting the fact that the presence of diuron herbicide disrupts algal photosynthetic metabolic activity, thereby affecting oxygen production rates. Harguinteguy et al. [34] utilized *Myriophyllum aquaticum* to monitor heavy metal ion concentrations in rivers for four consecutive months, finding that the plant could accumulate heavy metal ions and proposing its use for early monitoring of heavy metal water pollution. The same research group also found that *Potamogeton pusillus* could be used for heavy metal ion monitoring [35].

Védrine et al. [36] designed an optical biosensor for water pollutant detection using *Chlorella vulgaris* microalgae. The microalgae were encapsulated on quartz microfiber filter surfaces, and the effects of herbicides on chlorophyll fluorescence were studied to detect concentrations of five pesticides: cresol, atrazine, simazine, isoproturon, and diuron. Results showed detection capability at concentrations >1 µg/mL, with detection limits of 5 µg/L, 0.255 µg/L, 0.5 µg/L, 0.025 µg/L, and 0.025 µg/L, respectively. Merkoçi et al. [37] used bismuth

as a precursor, employing the binding interaction between tissue and bismuth to immobilize mushroom tissue onto multi-walled carbon nanotube-modified screen-printed electrodes during electrochemical bismuth deposition, creating a phenol sensor. The sensor exhibited a linear response range of 2–200 µmol/L and a detection limit of 1.17 µmol/L.

Microbial Sensors in Water Environment Monitoring

Microbial sensors employ microorganisms as recognition elements, utilizing their metabolic processes in the presence of target analytes to achieve detection. Microorganisms can be single strains, microbial communities, or dead cells.

Detecting biochemical oxygen demand (BOD) in water by combining microorganisms is an important application direction for microbial sensors in water environment monitoring. Large amounts of organic pollutants in domestic and industrial wastewater can be decomposed by aerobic bacteria through biochemical processes, consuming substantial dissolved oxygen, disrupting the oxygen balance in water bodies, and causing hypoxic death of fish and other aquatic organisms. Since organic matter composition in water is complex, the oxygen consumed by organic matter under certain conditions is typically used to indirectly represent organic content. BOD can relatively represent the amount of biodegradable organic pollutants, aligning with actual water self-purification conditions and thus having more practical significance in water quality assessment. Traditional BOD detection methods mainly include the five-day culture method, manometric method [38], activated sludge aeration degradation method [39], coulometric method [40], and elevated temperature method [38]. However, these methods suffer from long determination periods, complex operation, unsuitability for field monitoring, and inability to reflect water quality conditions in real time.

In 1977, Karube et al. [41] first modified microorganisms from soil on electrodes to measure BOD in sewage, achieving online rapid BOD detection. Microbial BOD sensors typically consist of microbial membranes and oxygen electrodes, directly detecting oxygen concentration changes caused by microbial biodegradation of organic matter. They offer advantages of simplicity, easy miniaturization, integration, and convenient use. Since then, numerous studies on using microorganisms for BOD detection have been reported.

The Environmental Microbiology Laboratory of the Wuhan Institute of Virology, Chinese Academy of Sciences, pioneered BOD microbial sensor research in China. Zhang et al. [42] screened *Pseudomonas* strains with broad metabolic spectra and degradation capabilities as working strains for BOD microbial sensors in 1986, enabling BOD determination within 15 minutes and suitability for BOD measurement in toxic industrial wastewater. The intelligent BOD instrument developed by this team in 1987 could be applied at petrochemical wastewater treatment plants and other field sites, winning the Chinese Academy of Sciences Science and Technology Progress Award (Third Prize) in 1989, thereby

providing important impetus for BOD microbial sensor research and development.

The Dong Shaojun team at the Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, has made important contributions to both fundamental and applied research on BOD microbial sensors. Since 2003, this team has published over 20 papers related to BOD microbial sensors and developed BOD measurement instruments for field application at Changchun Environmental Monitoring Center Station and other locations. BOD microbial sensor research involves materials and methods for microbial immobilization, such as sol-gel materials [43], organic-inorganic hybrid materials [44], nanotubes [45], and graphene [46], as well as rapid detection methods [47] and online monitoring systems [48,49]. Achieving effective microbial immobilization, improving BOD sensor stability and lifetime, shortening detection time, and enabling rapid BOD detection and online monitoring are directions of research interest.

Wang et al. [50] used immobilized microbial cell (IMC) beads as recognition elements for BOD measurement, which effectively reduced mass transfer resistance and improved sensitivity and stability compared with traditional biosensitive membranes. IMC beads maintained biological activity for approximately 70 days at a detection frequency of eight times per day, enabling rapid BOD measurement. Liu et al. [49] constructed a BOD flow detection system using potassium ferricyanide as a mediator and carbon fiber felt as a microbial immobilization material. The system could detect BOD within 30 minutes with stability up to two months. Hooi et al. [51] developed a BOD detection microbial sensor by immobilizing microorganisms on ultramicroelectrodes using calcium alginate. The sensor effectively shortened response time and achieved rapid BOD detection by leveraging the fast diffusion mass transfer characteristics of ultramicroelectrodes. Kashem et al. [52] constructed an optical BOD biosensor by implanting yeast into an oxygen-sensitive membrane structure wrapped with polyethylene-polypropylene (PE-PP). The PE-PP membrane could eliminate interference from environmental samples and offered advantages of low reagent consumption and short testing time (5 minutes). Wang et al. [53] prepared a membrane-free BOD microbial sensor based on magnetically modified microorganisms [Figure 3: see original paper]. The study used *Bacillus subtilis* as the microorganism for organic matter metabolism, adsorbing iron(III) oxide nanoparticles (positively charged) onto *Bacillus subtilis* surfaces (negatively charged) to form magnetic microorganisms. Using ultramicroelectrode arrays and nano-palladium/reduced carboxyl graphene-modified ultramicroelectrode arrays as transducers, a magnetic substrate was designed at the bottom of the ultramicroelectrode array. Magnetic microorganisms were immobilized on the ultramicroelectrode array surface as a sensitive membrane through magnetic fields, with external magnetic field control enabling sensitive membrane renewal. The sensor features simple preparation and easy renewal, facilitating on-site rapid BOD detection.

[Figure 3: see original paper] Membrane-free BOD microsensor based on mag-

netic microorganisms [53]
(a) BOD microsensor design; (b) Ultramicroelectrode array structure and photograph; (c) Schematic diagram and SEM image of *Bacillus subtilis* functionalized with iron(III) oxide; (d) Teflon substrate design and photograph; (e) Photograph of BOD microsensor

Several Key Technologies for Water Quality Biosensors

Water environment monitoring sensors face complex detection objects and environments. Developing biosensors for on-site, real-time, and online water quality detection involves numerous scientific issues and technical challenges, requiring solutions for sensor sensitivity, selectivity, stability, as well as device and system miniaturization, low power consumption, and long-term autonomous operation. The authors provide specific analysis of related issues in an upcoming publication , with emphasis here on three key technological aspects.

Biomaterial-Based Enrichment Techniques For certain highly toxic pollutants with low standard limits, direct detection is challenging. Pre-enrichment methods can enable trace detection. Common liquid-phase enrichment methods include extraction [54], adsorption [55], ion exchange [56], membrane separation [57], and biochemical methods [58]. These methods generally suffer from expensive equipment/reagents and environmental unfriendliness [59]. In recent years, with rapid development in biology and biological characterization methods, biochemical enrichment methods represented by microbial adsorption have received widespread attention. By leveraging complexation, ion exchange, and physical adsorption between biological materials and metal ions, biological materials can serve as adsorption enrichment materials in sensors [60–63]. For example, Fiol et al. [64] incorporated yohimbe stems and grape stems into PVC membranes as bioadsorption materials for pre-enrichment of Hg^{2+}.

Biosensor Miniaturization With the development of micro-nano fabrication technology and nanoscience, and the need for constructing wireless sensor networks for water environment monitoring, miniaturization of water quality monitoring sensors has become a development trend. Micro-nano fabrication technology can promote sensor miniaturization and batch manufacturing, improving sensor consistency, while nanotechnology can effectively ensure and enhance sensor sensitivity and selectivity during chip miniaturization.

Biosensor miniaturization offers several advantages [65–67]: (1) Reduced consumption of expensive biofunctional materials, effectively lowering research and development costs; (2) Enables microanalysis, reducing required reagent quantities and waste liquid generation; (3) Facilitates system integration, benefiting the formation of biosensor systems for wireless sensor network monitoring of water environments. However, numerous technical challenges remain in biosensor miniaturization, including integration of sensitive elements, biomaterial immobilization and activity maintenance, and pretreatment of water samples.

Renewable Immobilization Methods for Biological Sensitive Materials
The type, quantity, immobilization method, and metabolic activity of biofunctional materials selected for preparing biological sensitive membranes significantly impact sensor performance. Excellent biosensors require both confining bio-sensitive materials within a defined space without loss and maintaining their inherent biological activity. Existing immobilization technologies include entrapment, adsorption, and cross-linking methods, which all suffer to varying degrees from activity reduction, cross-linking negative effects, and difficult membrane renewal.

Recent research has emerged on renewable immobilization methods for biological sensitive materials. The authors' team investigated magnetically functionalized cell technology for biosensor construction [53]. Magnetically functionalized cells can be enriched around magnet channels, achieving patterned cell immobilization regions through magnet channel design to improve the quantity and quality of cells fixed on sensitive surfaces. When the magnet is removed, appropriate washing enables re-immobilization, effectively maintaining sensitive membrane activity and opening new methods for renewable sensor membranes. Both yeast and algae can be magnetically functionalized with iron(III) oxide nanoparticles. Through multicenter adsorption, polymer-modified nanoparticles (positively charged) adsorb onto biological entity surfaces (negatively charged), and the magnetized yeast or algal cells are then immobilized on sensor interfaces to form sensitive membranes. The entire process is time-saving and more efficient than traditional methods [68].

Conclusion

Research on biosensors for water environment monitoring continues to expand and deepen, demonstrating promising development and application prospects. Currently, due to stability and consistency issues with biofunctional material activity, most biosensors still struggle to meet the demands of long-term online monitoring applications, awaiting further research and breakthroughs in principles, methods, and technologies.

It is anticipated that biosensors will achieve greater innovative development when combined with emerging technologies such as micro-nano technology and microfluidics, enabling water environment monitoring biosensors with small size, rapid response, high sensitivity, strong anti-interference capability, and long service life. This will have important implications for water environment and resource monitoring and protection.

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